



IGF-I RIA KIT

REF	10 IGF50	REF	10 IGF100
Σ	50	Σ	100



WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

The manufacturer disclaims any and all implied warranties of merchantability, fitness for use or implied utility for any other purposes. Any and all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

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INTENDED USE

The IGF-I RIA has been designed for the quantitative *in vitro* diagnostic measurement of IGF-I (Insulin-like growth factor I) in serum or plasma.

PRINCIPLES OF THE RIA

The RIA is a double antibody radioimmunoassay system. The method includes a simple extraction step in which IGF-I is first separated from its binding protein in serum. After the short extraction procedure, the analyte competes with ¹²⁵I labelled tracer antibody for binding to a constant amount of antibody.

A second antibody coupled to magnetisable polystyrene particles (Separation Reagent) is used to separate antibody-bound from free ¹²⁵I labelled tracer antibody. Following sedimentation, the supernatant is discarded and the pellet containing the bound radioactivity is counted using a gamma counter. The concentration of the analyte is inversely proportional to the bound radioactivity in the pellet. Counts from the calibrators are plotted and samples are read from the constructed calibrator curve.

REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 50 tests and 100 tests (in parentheses). The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

IGF-I: Tracer

1 vial Ref # IGI1
(1 vial Ref # IGI2)
5.5 (10.5) mL ¹²⁵I des labelled IGF-I ($\leq 135\text{kBq}$ / $\leq 270\text{kBq}$) in BSA PBS buffer containing a red dye. Contains Bronidox L, 0.05% w/v. Ready to use.

IGF-I: Antiserum

1 vial Ref # IGA1
(1 vial Ref # IGA2)
5.5 (10.5) mL containing rabbit IGF-I antiserum diluted in BSA PBS buffer and a blue dye. Bronidox L, 0.05% w/v. Ready to use.

Separation Reagent

1 vial Ref # SEP1
(1 vial Ref # SEP2)
13 (26) mL containing goat anti-rabbit antibody coupled to magnetisable polystyrene particles in BSA PBS buffer. Contains sodium azide, 0.1% w/v. Resuspend gently before use.

Acid-Ethanol Solution

1 vial Ref # IGAE1
(1 vial Ref # IGAE2)
20 (40) mL of 87.5% ethanol in diluted HCl. Keep well stoppered. Ready to use.

Neutralising Solution

1 vial Ref # IGNS1
(1 vial Ref # IGNS2)
20 (40) mL of Tris-phosphate buffer. Ready to use.

Wash Concentrate

1 vial Ref # HW1
10 mL of a 15 x concentrated wash solution. Contains sodium azide, 1.5 % w/v. To be diluted before use.

IGF-I: Calibrators

7 vials Ref # IGSA-G
2.0 mL in Calibrator A and 0.5 mL in Calibrator B-G, each in BSA PBS. Contains Bronidox L, 0.05% w/v. Lyophilized.

IGF-I: Control Serum

1 vial Ref # IGC1
0.5 mL of human serum. Contains sodium azide, 0.1% w/v. Lyophilized.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations. **Specimens, Calibrators and Controls**

The source material of the calibrators and controls has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all.

However it is recommended that all samples be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains sodium azide and Bronidox L as a preservative. As reagents contain a potentially toxic preservative, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Radioactive Material

The tracer contains radioactive material.

SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulants heparin or EDTA can be employed without sacrificing accuracy.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly.

Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled or deionised water
- * Disposable plastic test tubes with caps 12 x 75 mm
- * Precision pipettes
- * Repeating pipettes
- * Vortex mixer
- * Roller Bench
- * Timer
- * Refrigerated centrifuge capable of 2000 x g
- * Magnetic Rack
- * Absorbent paper
- * Gamma counter

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. All assay steps should be performed without interruption.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.

The gamma counter and all pipettes used should be calibrated appropriately before use.

If a centrifuge does not attain at least 2000 x g, an unstable pellet may result. Therefore the centrifuge time must be increased.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 3 months.

Calibrators and Controls

To reconstitute the lyophilized calibrators and control, add the volume of deionized water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion. Exact concentrations and range determined lot-to-lot are stated on a separate label inside the kit. After reconstitution the calibrators and control should be stored at -20°C.

Separation Reagent

Mix well on a roller bench before use.

Sample Preparation / Extraction Procedure

1. Label extraction tubes, one for each specimen and controls.
2. Pipette 100 µL of specimen into extraction tubes.
3. Pipette 400 µL of Acid-Ethanol Solution. Cap and vortex. Leave at room temperature (20-25°C) for 30 minutes.
4. Centrifuge all tubes for 20 minutes at 2000 x g in a refrigerated centrifuge (4°C).
5. Label more test tubes, one for each specimen/control.
6. Carefully transfer a 50µL aliquot of each supernatant into appropriate extraction tubes.
7. Pipette 500µL of Neutralising Solution to each tube. Vortex.

This is the neutralised sample extract.

Protocol

Radioimmunoassay Procedure

1. Assemble and label test tubes in duplicate according to the number of tests required. Include Total Counts (TC), Non-Specific Binding (NSB), calibrators, extracted controls/specimens.
2. Pipette 200 µL of Calibrator A in duplicate into the NSB tubes.
3. Pipette 100 µL of sample (calibrator, extracted control/specimen) in duplicate into the appropriate tubes.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction.

1. Determine the average cpm for duplicate tubes.
2. Plot the calibrator curve on a semi-log or log-linear graph paper using the method below:

Use the following formula to calculate %B/T:

$$\%B/Bo = \frac{\text{cpm (Sample)} - \text{cpm (NSB)}}{\text{cpm (Calibrator A)} - \text{cpm (NSB)}} \times 100$$

3. Plot %B/Bo on the y axis versus the stated concentrations of the calibrators.
4. Read samples directly off the calibrator curve as ng/mL.

MODEL CALCULATIONS

ID	Ave cpm	%B/Bo	IGF-I (ng/mL)
TC	89279		
NSB	628		
0	15922	100.0	
40	13493	84.1	46
100	10898	67.2	107
250	7078	42.2	273
500	4326	24.2	578
1000	2638	13.1	1129
2000	1753	7.4	2311
Control	7804	46.9	227
Sample 1	10285	63.1	126

CALIBRATION

The calibrators supplied in this kit are calibrated to (IRR IGF-I, 87/518 Est 1988) They are labelled ng/mL.

Conversion of calibrator units may be made using the following relationship:

$$1 \text{ U/mL IGF-I} = 240 \text{ ng/mL IGF-I} = 31.38 \text{ nmol/L IGF-I}$$

4. Pipette 100 µL of IGF-I Tracer (red) into all tubes.
5. Pipette 100 µL of IGF-I Antiserum (blue) to all tubes except NSB and TC.
6. Vortex tubes gently and incubate 2 hours (or overnight stationary) at room temperature (20-25°C). All tubes should be purple except NSB and TC tubes.
7. At the end of the incubation period, pipette 250 µL of the thoroughly mixed Separation Reagent into all tubes except TC and vortex. Set TC tubes aside, and incubate for 15 minutes stationary at room temperature (20-25°C).
- 8a. To Separate antibody from unbound label, place test tubes into magnetic separation rack and ensure all test tubes are in contact with magnetic baseplate. Leave for 2 minutes.
- 8b. Do not remove rack from magnetic baseplate. Decant the supernatant and keep magnetic baseplate inverted. Tap the tubes firmly onto absorbent paper and blot the rims to remove all residual supernatant.
- 8c. Remove rack from magnetic baseplate. Pipette 500 µL of wash solution to all tubes. Vortex, sediment on magnetic baseplate, decant and drain tubes.
9. Count the tubes for one minute using a gamma counter. Counting longer will reduce statistical counting error. Record the cpm of each tube.
10. Calculate results.

LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

Samples that contain appreciable background radioactivity should not be used. Any suspect samples should be screened for radioactivity before performing the assay and should be held until the radioactivity has decayed, or a new sample requested.

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range was obtained by assaying serum samples from healthy individuals and is given as a guide only:

Age (Yrs)	n	Mean	SD	Median	5th Percentile	95th Percentile
0 (Male & Female)	28	68.5	62.4	58.5	19.8	108
1 - 3	40	98.6	58.1	88.1	39.7	229
4 - 6	29	117.0	67.1	97.9	45.3	247
7 - 9	39	203.0	107.0	184.0	71.7	450
10 - 12	38	327.0	150.0	324.0	122.0	521
13 - 15	52	388.0	140.0	388.0	158.0	605
16 - 18	27	461.0	109.0	454.0	282.0	641
19 - 30	106	294.0	96.7	282.0	152.0	424
31 - 40	55	220.0	75.1	215.0	118.0	326
41 - 50	68	193.0	65.2	196.0	108.0	297
51 - 60	61	170.0	58.9	163.0	82.2	268
61 +	25	165.0	54.6	157.0	86.8	241

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (ng/mL)	%CV
A	22	70.4 ± 3.8	5.4
B	22	123.0 ± 5.2	4.2
C	22	225.0 ± 7.7	3.4

Inter-assay Precision

Sample	n *	Mean ± 2SD (ng/mL)	%CV
G	36	77.6 ± 4.6	5.9
H	36	135.0 ± 7.5	5.6
I	36	227.0 ± 9.8	4.3

* duplicate, same day option

Specificity

Analyte	Concentration Assayed	Apparent IGF-I Result
Human GH	1000 nmol/L	undetectable
Prolactin	25000 mIU/L	undetectable
IGFBP-1	1000 ng/mL	undetectable
IGFBP-2	1000 ng/mL	undetectable
IGF-II	4000 ng/mL	undetectable

Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	IGF-I(ng/mL) Observed	IGF-I(ng/mL) Expected	% Recovery
1	197	203	97.0
2	241	258	93.4
3	595	602	98.8

Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	IGF-I (ng/mL) Observed	IGF-I (ng/mL) Expected	% Recovery
Neat	456		
1/2	233	228	102.0
1/4	108	114	94.7

Sensitivity

The sensitivity, defined as that concentration of analyte corresponding to two standard deviations from the mean of the dose response variable of the zero calibrator (n=20; measured in 9 assays), is typically less than 1.0 ng/mL. In terms of the actual concentration of IGF-I, the sensitivity is 0.018 ng/mL.

Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

ORDERING INFORMATION

The IGF-I RIA is manufactured by:
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